group represented by the foumula (1) given above and has a purity as high as 80 % or more, preferably as high as 90 % or more. The high purity polysaccharide according to the present invention can form a finely dispersed colloid solution by its cohesive nature due to the hydrophobic group represented by the formula (1) and reveals thus an ability for building up polymer micelles of a core/shell type.

The high purity polysaccharide containing hydrophobic group according to the present invention can be utilized as coating material for coating drug carrier having encapsulated therein a drug. It can be used as the coating material for coating drug carrier, such as liposome microcapsule, microsphere, O/W emulsion and erythrocyte ghost. The high purity polysaccharide containing hydrophobic group can be used for such medical materials safely, since it has scarce contents of by-products and of the unsubstituted polysaccharide and is highly pure.

As described above, the process for producing the high purity polysaccharide containing hydrophobic group according to the present invention permits to produce a high purity product of polysaccharide containing hydrophobic group having scarce content of impurities, such as unsubstituted polysaccharide and sterol dimer, in an easy and efficient manner, since it includes a step of purification using a solvent based on ketone. A more highly pure product of polysaccharide containing hydrophobic group can be produced by incorporating, in combination, a purification by an

ultracentrifugation or a purification with an aprotic polar solvent.

The high purity polysaccharide according to the present invention has a high purity due to its production process as described above, so that it can be used as a medical material safely.

Below, the present invention will further be described more concretely by way of examples.

EXAMPLE 1-1

《 Synthesis of N-(6-isocyanatohexyl)cholesteryl carbamate 》

In an eggplant type flask of 1 liter capacity, there are charged 25 g (0.065 mole)of cholesterol and thereto were added 300 ml of toluene to dissolve it, whereto 17 ml (0.12 mole) of triethylamine were added. To this mixture, there were added 161 g (0.96 mole, 14.8 eq.) of hexamethylene diisocyanate dissolved in 300 ml of toluene and the resulting mixture was subjected to reaction at 80 °C under a nitrogen atmosphere for about 6 hours. After termination of the reaction, toluene and the excess of hexamethylene diisocyanate were removed under a reduced pressure. By standing the resulting yellowish oily residue still at room temperature overnight, pale yellow crystals were formed. The crystals were taken out and introduced into about one liter of hexane and the mixture was shaken vigorously, whereupon the supernatant was removed by decantation. This washing procedure was repeated four times, whereupon the product was dried under a reduced pressure for three hours at room

temperature, whereby a white solid product (crystals) was obtained. Yield: 18.25 grams (50.9 %)

The results of analysis of this product by ^{1}H-NMR and IR were as given below.

^{1}H-NMR: δ ppm, in $CDCl_3$, TMS

0.68 - 2.35 (m, 43H)

1.34 - 1.55 (m, 8H)

3.14 - 3.18 (m, 2H)

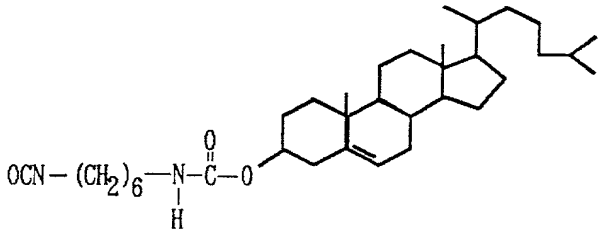
3.27 - 3.32 (t, J=6.6 Hz, 2H)

4.4 - 4.6 (m, 2H)

5.38 (m, 1H)

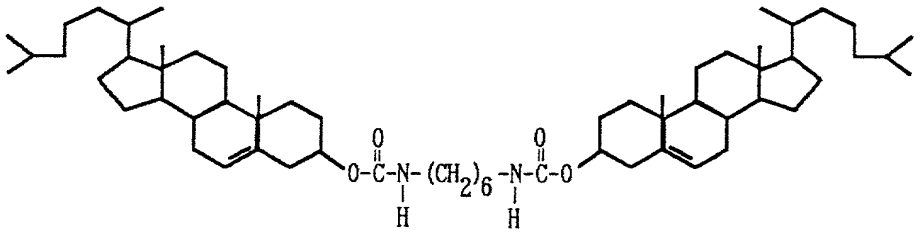
IR (KBr, cm^{-1}):3260, 2320, 1680, 1130

From these data, it was confirmed that the product obtained is N-(6-isocyanatohexyl)cholesteryl carbamate represented by the following formula (4a):



(4a)

On the other hand, the white crystls obtained were examined by a thin layer chromatography by developing it using Preparative TLC (supplied from Merck AG, Silika gel 60 F_{254}, developer: hexane/ethyl acetate = 2/1), whereby existence of the by-produced cholesterol dimer (Rf = 0.65) represented by the following formula (5a) was confirmed. The band for the cholesterol dimer on the TLC was subjected to extraction with acetone and the extract was analyzed, whereby it was confirmed that the cholesterol dimer was contained in the white crystalline product in an amount of 8 % by weight.



(5a)

EXAMPLE 1-2

《 Synthesis of pullulan-cholesterol derivative (CHP) 》

In an eggplant type flask of 1 liter capacity,

there are charged 40 g (248 mmol as anhydrous glucose unit) of pullulan (average molecular weight: 108,000) and 420 ml of dimethyl sulfoxide (sometimes abbreviated as DMSO) were added thereto and the mixture was agitated at 80 °C under a nitrogen atmosphere to dissolve it. To this mixture, a solution of 1.78 g (3.21 mmol) of N-(6-isocyanotohexyl)cholesteryl carbamate synthesized in EXAMPLE 1-1 dissolved in 31.6 g (0.40 mol) of pyridine was added and the mixture was subjected to reaction at 90 °C for 3 hours.

After termiantion of the reaction, dimethyl sulfoxide was distilled off and the resulting oily residue was dropped into 6 liters of acetone to form a precipitate to purify the product. After removal of the supernatant, 4 liters of acetone were added to the resulting precipitate and the mixture was stood still overnight at room temperature. The precipitate was collected by filtration and was dried under a reduced pressure. The so-obtained solids were dissolved in dimethyl sulfoxide and the solution was charged in a dialysis bag (Spectra/Por3, a product of the frim Spectropor, an exclusion molecular weight of 3,500) and was subjected to a dialysis against distilled water for one week. 1.5 liters of the resulting polymer solution were treated by freeze-drying in an ordinary manner, whereby a white solid matter (in the following, denoted occasionally as "acetone-purified product") was obtained. Yield: 31.7 g (76.2 %)

The results of analyses of this acetone-purified product by ^{1}H-NMR and IR were as given below.

^{1}H-NMR: δ ppm, DMSO-d_6/D_2O = 20/1, vol.

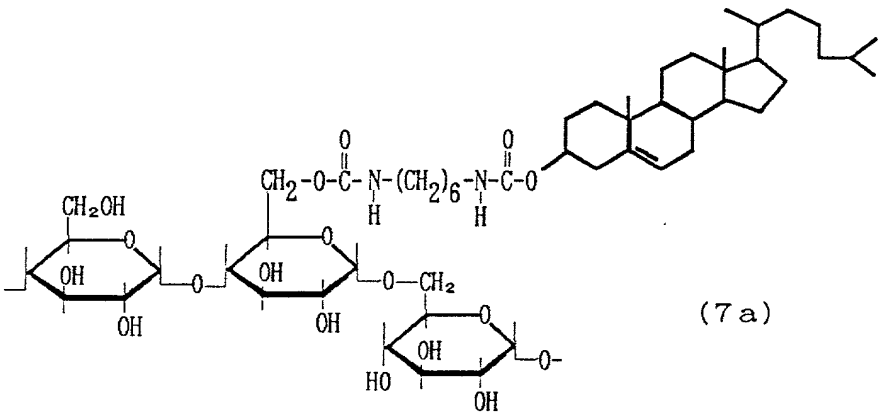
0.68 - 2.40

2.60 - 4.60

4.70 - 5.30

IR (KBr, cm^{-1}): 1680, 1180 - 900

From these data, it was confirmed that the product obtained is a pullulan/chlosterol derivative (abbreviated hereinafter sometimed as CHP) represented by the following formula (7a):



(7a)

On the other hand, the above product purified with acetone were analyzed by a thin layer chromatography using Preparative TLC {supplied from Merck AG, Silika gel 60 F_{254}, developer: hexane/ethyl acetate (2/1)}, whereby existence of the by-produced cholesterol dimer (Rf = 0.65) represented by the formula (5a) was not recognized. Further, the acetone-purified product was analysed by ^{1}H-NMR and the content of the cholesterol dimer was calculated from the protone ratio. From the result, existence of the cholesterol dimer was not recognized. Therefore the content of the cholesterol dimer represented by the

formula (5a) in the acetone-purified product is 0 % by weight.

The acetone layer used for the purification was collected and the amount of cholesterol dimer contained therein was estimated. It was found that the amount of cholesterol dimer contained in the acetone layer was 0.140 gram. Since the amount of cholesterol dimer contained in the raw material of 1.78 grams (3.21 mmol) of N-(6-isocyanatohexyl)cholesteryl carbamate is calculated to be 0.142 gram (It had been confirmed that the content of cholesterol dimer in the product of EXAMPLE 1-1 was 8 % by weight), the rate of removal of the cholesterol dimer is calculated from these data. Result is recited in Table 1.

The ^{1}H-NMR spectrogram of the resulting target compound, i.e., pullulan-cholesterol derivative represented by the formula (7a), is given in Fig. 1. From the integration value of the peak area of this ^{1}H-NMR spectrogram, the proportion of introduction of the cholesterol groups into pullulan of the pullulan-cholesterol derivative is calculated by the following calculation equation (A):

$$(100 + 2x)/51x = b/a \quad \cdots\cdots (A)$$

in which the symbols denote:

a: the peak area of the cholesterol group (δ = 0.68 - 2.40)

b: the peak area of pullulan (δ = 4.70 - 5.30)

x: proportion of substitution with cholesterol group per 100 monosaccharide units.

From the calculation, it is found that the

proportion of substitution with cholesterol group in the pullulan-cholesterol derivative represented by the above formula (7a) is 1.1 groups per 100 monosaccharide units.

COMPARATIVE EXAMPLE 1

《 Test of reprecipitation with ethanol》

A pullulan-cholesterol derivative was synthesized by the same procedures as in EXAMPLE 1-2. After termination of the reaction, the purification was performed by reprecipitation with ethanol, whereby a pullulan-cholesterol derivative was obtained.

The analysis of the product purified with ethanol was performed using Preparative TLC in the same manner as in EXAMPLE 1-2. From the result, existence of the cholesterol dimer (Rf = 0.65) represented by the formula (5a) was confirmed. The content of the cholesterol dimer in the product purified with ethanol was calculated from ^{1}H-NMR analysis as in EXAMPLE 1-2. The result showed that the cholesterol dimer is contained in the product in an amount of 0.4 % by weight.

The ethanol layer used for the purification was collected and the amount of the cholesterol dimer contained therein was estimated, which showed that the content was 0.016 gram. From this, the rate of removal of the cholesterol dimer was calculated in the same manner as in EXAMPLE 1-2. The result is recited in Table 1.

Table 1

	EXAMPLE 1-2	COMP. EXAMPLE 1
Solvent for reprecipitation	Acetone	Ethanol
Colesterol dimer content (wt. %)	0	0.4
Cholesterol dimer removal wt. %	98.6	11.3

From Table 1, it is confirmed that the cholesterol dimer can be removed almost completely by purifying the product by reprecipitation using acetone as the reprecipitation solvent.

EXAMPLE 1-3

《 Purification of pullulan-cholesterol derivative (CHP)》

To 40 mg of the pullulan-cholesterol derivative synthesized in EXAMPLE 1-2, 20 ml of pure water were added and the mixture was subjected to an ultrasonic wave irradiation by a sonicator of a probe-type (TOMY, a unit supplied by the firm URP, with a probe outer diameter of 5 mm) for 30 minutes at 40 W. During the irradiation, the temperature of the aqueous mixture was maintained at 4 °C by cooling the vessel from outside with ice water.

Samples of each 10 ml of the ultrasonic wave-irradiated aqueous mixture were collected in centrifuge tubes and were subjected to a centrifugation at 55,000 G for 10 hours at 25 °C. A phase separation was brought about, wherein the unsubstituted pullulan (unreacted pullulan) was gathered in the supernatant and the pullulan-cholesterol derivative (CHP) was separated in

the lower layer.

The samples collected before and after the ultrasonic wave irradiation, respectively, were analysed by SEC (size exclusion chromatography). The conditions in the SEC were as given below. The results are shown in Figs. 2(a) and 2(b), respectively.

Apparatus used:		TOSOH HPSEC SYSTEM (trademark, of Tosoh K.K.)
Column	:	TSK-gel G4000SWXL (trademark, of Tosoh K.K.)
Eluent	:	0.02 % NaN_3 in deionized water
Flow rate	:	0.5 ml/min.
Temperature	:	35 °C
Detector	:	RI (differential refractometer)

Calculated from the peak area of Fig. 2(b), it is found that pullulan of which molecular weight is lower (unreacted pullulan) is contained in the product purified with acetone in an amount of about 5 % by weight. The supernatant of ultracentrifugation was subjected to an SEC analysis. The result is shown in Fig. 2(c). The gelled mass (precipitate) in the lower layer of the centrifugation was caused to swell again with water and was then subjected to an ultrasonic wave treatment in the same manner as above, whereupon the so-ultrasonicated solution was examined by SEC analysis. The result is shown in Fig. 2(d). From these results, it is confirmed that, in the supernatant of the ultracentrifugation, almost 100 % by weight of the lower molecular weight pullulan (unreacted pullulan) as impurity have been removed and that the

precipitate has no content of the lower molecular weight pullulan.

From the above results, it was confirmed that the pullulan-cholesterol derivetive (CHP) represented by the formula (7a) was obtained at a high purity. The results are summarized in Table 2.

Table 2

Content of CHP in acetone-purified product (wt. %)	95
Content of CHP in ultracentrifug.-purified product (wt. %)	100
Content of unsubstituted pullulan (wt. %)	0
Content of cholesterol dimer (wt. %)	0

EXAMPLE 2-1

《 Synthesis 1 of mannan-cholesterol derivative (CHM) 》

By following the same procedures for the reaction as in EXAMPELE 1-2, a commercial product of mannan (a product of the firm Sigma) and N-(6-isocyanatohexyl)cholesteryl carbamate were brought into rection. The charged amount of each starting material was as given below:

1) Mannan (Mw = 85,000): 26.2 g (162 mmol as anhydrous mannose unit)
2) N-(6-isocyanatohexyl)cholesteryl carbamate: 1.08 g (1.95 mmol)
3) Pyridine: 19.2 g (243 mmol)
4) Dimethyl sulfoxide: 320 ml

After termination of the reaction, purification

was performed by reprecipitation with acetone solvent. Then, the product was subjected to a dialysis, followed by a freeze-drying, whereby 21.5 grams (yield = 79.5 %) of a white solid matter was obtained.

The results of analyses of the above acetone-purified product by ^{1}H-NMR and IR are given below:

^{1}H-NMR: δ ppm, DMSO-d_6/D_2O = 20/1, vol.

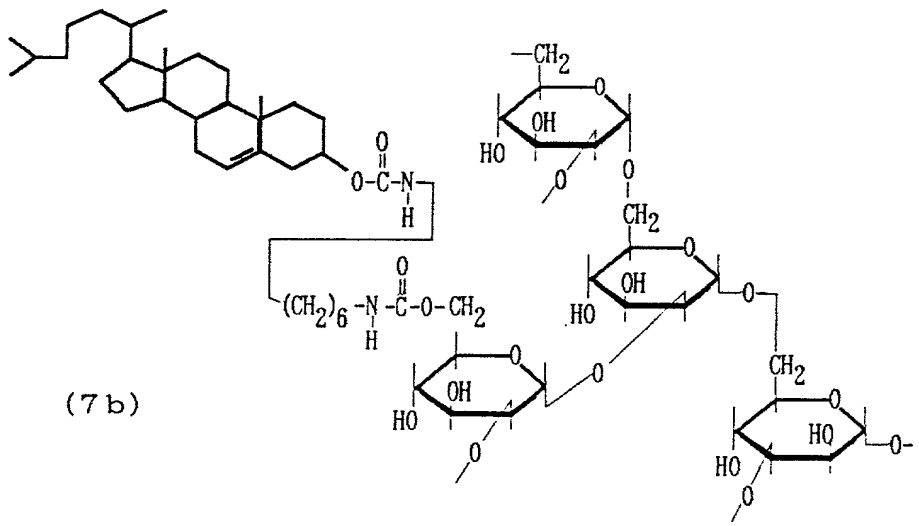
0.68 - 2.40

2.60 - 4.60

4.60 - 5.40

IR (KBr, cm^{-1}): 1680, 1180 - 900

From these data, it was confirmed that the product obtained is a mannan/chlosterol derivative (CHM) represented by the following formula (7b):



(7b)

On the other hand, the above product purified with acetone was analyzed using Preparative TLC in the same manner as in EXAMPLE 1-2, whereby existence of the cholesterol dimer (Rf = 0.65) represented by the formula (5a) was not recognized. Further, the acetone-purified

product was analyzed by ^{1}H-NMR and the content of the cholesterol dimer was calculated from the proton ratio. From the result, existence of the cholesterol dimer was not recognized. Therefore the content of the cholesterol dimer represented by the formula (5a) in the acetone-purified product is 0 % by weight.

^{1}H-NMR spectrogram of the compound represented by the above formula (7b) obtained in the manner described above is shown in Fig. 3. The proportion of introduction of the cholesteryl group into mannan in the mannan-cholesterol derivative is calculated in the same way as in EXAMPLE 1-2. From the result, it is found that the proportion of substitution with cholesteryl group is 1.1 groups per 100 monosaccharide units.

Then, the acetone-purified product obtained as above was further purified by an ultracentrifugation in the same manner as in EXAMPLE 1-3 to remove the lower molecular weight mannan (unsubstituted mannan). The results are given in Table 3.

Table 3

Content of CHM in acetone-purified product (wt. %)	90
Content of CHM in ultracentrifug.-purified product (wt. %)	100
Content of unsubstituted mannan (wt. %)	0
Content of cholesterol dimer (wt. %)	0

EXAMPLE 2-2

《 Synthesis 2 of mannan-cholesterol derivative (CHM) 》

By following the same procedures for the reaction as in EXAMPELE 1-2, a commercial product of mannan (a product of the firm Sigma) and N-(6-isocyanatohexyl)cholesteryl carbamate were brought into rection. The charged amount of each starting material was as given below:

1) Mannan (Mw = 85,000): 5 g (31 mmol as anhydrous mannose unit)
2) N-(6-isocyanatohexyl)cholesteryl carbamate: 138 mg (0.25 mmol)
3) Pyridine: 3.7 g (47 mmol)
4) Dimethyl sulfoxide: 75 ml

After termination of the reaction, purification was performed by reprecipitation with acetone solvent. Then, the product was subjected to a dialysis, followed by a freeze-drying, whereby 4.05 grams (yield = 78.8 %) of a white solid matter was obtained.

The results of analyses of the above acetone-purified product by ^{1}H-NMR and IR are given below:

^{1}H-NMR: δ ppm, DMSO-d_6/D_2O = 20/1, vol.

0.68 - 2.40

2.60 - 4.60

4.60 - 5.40

IR (KBr, cm^{-1}): 1680, 1180 - 900

From these data, it was confirmed that the product obtained is a mannan/chlosterol derivative (CHM) represented by the above formula (7b):

On the other hand, the above product purified with acetone was analyzed using Preparative TLC in the same manner as in EXAMPLE 1-2, whereby existence of the

cholesterol dimer (Rf = 0.65) represented by the formula (5a) was not recognized. Further, the acetone-purified product was analyzed by ^{1}H-NMR and the content of the cholesterol dimer was calculated from the proton ratio. From the result, existence of the cholesterol dimer was not recognized. Therefore the content of the cholesterol dimer represented by the formula (5a) in the acetone-purified product is 0 % by weight.

^{1}H-NMR spectrogram of the mannan-cholesterol derivative (CHM) obtained in the manner described above is shown in Fig. 4. The proportion of introduction of the cholesteryl group into mannan in this compound is calculated in the same way as in EXAMPLE 1-2. From the result, it is found that the proportion of substitution with cholesteryl group is 0.8 group per 100 monosaccharide units.

Then, the acetone-purified product obtained as above was further purified by an ultracentrifugation in the same manner as in EXAMPLE 1-3 to remove the lower molecular weight mannan (unsubstituted mannan). The results are given in Table 4.

Table 4

Content of CHM in acetone-purified product (wt. %)	87
Content of CHM in ultracentrifug.-purified product (wt. %)	100
Content of unsubstituted mannan (wt. %)	0
Content of cholesterol dimer (wt. %)	0

EXAMPLES 3 to 6

《 Production of high purity polysaccharide containing steryl group 》

Using each a polysaccharide of natural origin, namely, xyloglucan (EXAMPLE 3), amylose (EXAMPLE 4), dextran (EXAMPLE 5) and a synthetic polysaccharide, i.e. hydroxyethyl cellulose (EXAMPLE 6), each a high purity polysaccharide-cholesterol derivative was obtained by similar procedures for reaction as those in EXAMPLES 1-2 and 1-3.

The proportion of introduction of cholesteryl group, the rate of removal of the cholesterol dimer (weight %) and the content thereof (weight %) were estimated in accordance with corresponding analytical techniques. The content of the unsubstituted polysaccharide was also determined each before and after the ultracentrifugation. The results are summarized in Table 5.

Table 5

	Example			
	3	4	5	6
Starting polysaccharide	Xyloglucan	Amylose	Dextran	Hydroxyet. cellulose
Number of cholesteryl groups in the product [1]	1.2	0.8	1.3	1.0
Purity of the acetone purified product (wt. %)	90	82	88	92
Purity of ultracentr.-purified product (wt. %)	100	100	100	100
Impurity				
Content of cholesterol dimer (wt. %)	0	0	0	0
Rate of removal of cholesterol dimer (wt. %)	98.4	98.8	98.6	98.4
Content of unreacted polysaccharide (wt. %)				
before ultracentrifugation	10	18	12	8
after ultracentrifugation	0	0	0	0

Note: 1) Number of cholesteryl groups introduced per 100 monosaccharide units in the product.

From Table 5, it is seen that cholesteryl group can be introduced in a similar way into other polysaccharides than pullulan and mannan.

EXAMPLE 7-1

《 Synthesis of N-(6-isocyanatohexyl)stearyl carbamate (synthesis of stearylpullulan)》

In an eggplant type flask of 1 liter capacity, there are charged 3.48 g (12.9 mmol) of stearyl alcohol and thereto were added 50 ml of toluene to dissolve it, whereto 2.04 g (25.8 mmol) of pyridine were further added. To this mixture, there were added 30 g (178 mmol, 14.8 eq.) of hexamethylene diisocyanate dissolved in 50 ml of toluene and the resulting mixture was subjected to reaction at 80 °C under a nitrogen atmosphere for about 3 hours. After termination of the reaction, toluene and the excess of hexamethylene diisocyanate were removed under a reduced pressure, whereby a pale yellow crystals were formed. The crystals were taken out and introduced into about one liter of hexane and the mixture was shaken vigorously, whereupon the supernatant was removed by decantation. This washing procedure was repeated four times, whereupon the product was dried under a reduced pressure for three hours at room temperature, whereby 2.75 g of a white solid product (crystals) were obtained (yield: 48.7 %).

The result of analysis of this product by ^{1}H-NMR was as given below.

^{1}H-NMR: δ ppm, in $CDCl_3$, TMS

0.88 (t, d = 6.8 Hz, 3H)

1.10 - 1.65 (m, 40H)

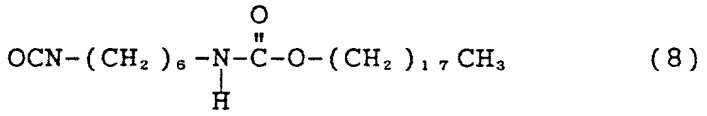
3.14 - 3.18 (m, 2H)

3.29 (t, J=6.6 Hz, 2H)

4.01 - 4.06 (m, 2H)

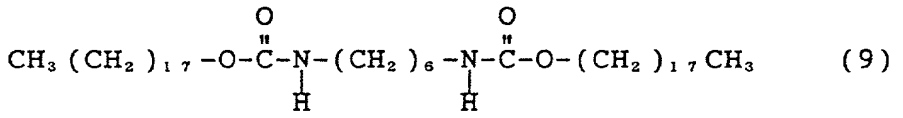
4.61 (m, 1H)

From these data, it was confirmed that the product obtained is N-(6-isocyanatohexyl)stearyl carbamate represented by the following formula (8):



$$OCN-(CH_2)_6-\underset{\underset{H}{|}}{N}-\overset{\overset{O}{\|}}{C}-O-(CH_2)_{17}CH_3 \qquad (8)$$

On the other hand, the above product purified with acetone were analyzed using Preparative TLC in the same manner as in EXAMPLE 1-1, whereby existence of a stearyl dimer (Rf = 0.68) represented by the following formula (9) was recognized. Further, the band for the stearyl dimer on the TLC was extracted with acetone and analysed quantitatively, whereby it was confirmed that the stearyl dimer was present in the white crystals in an amount of 3 % by weight.



$$CH_3(CH_2)_{17}-O-\overset{\overset{O}{\|}}{C}-\underset{\underset{H}{|}}{N}-(CH_2)_6-\underset{\underset{H}{|}}{N}-\overset{\overset{O}{\|}}{C}-O-(CH_2)_{17}CH_3 \qquad (9)$$

EXAMPLE 7-2

《 Synthesis of stearyl-pullulan derivative (STP) 》

In an eggplant type flask of 100 ml capacity, there are charged 2.0 g (12.3 mmol as anhydrous glucose unit) of pullulan (Mw = 108,000) and thereto were added 30 ml of dimethyl sulfoxide and the resulting mixture was agitated at 80 °C under a nitrogen atmosphere to

dissolve it. To this mixture, a solution of 70 mg (0.148 mmol) of N-(6-isocyanatohexyl)stearyl carbamate synthesized in EXAMPLE 7-1 dissolved in 1.47 g (14.6 mmol, 1.2 eq.) of pyridine was added and the mixture was subjected to reaction at 90 °C for 2 hours.

After termiantion of the reaction, dimethyl sulfoxide was distilled off under a reduced pressure and the resulting oily residue was dropped into 300 ml of acetone to form a precipitate to purify the product. After removal of the supernatant, 200 ml of acetone were added to the resulting precipitate and the mixture was stood still overnight at room temperature. The precipitate was collected by filtration and was dried under a reduced pressure. The so-obtained solids were dissolved in dimethyl sulfoxide and the solution was charged in a dialysis bag (Spectra/Por3, a product of the firm Spectropor, an exclusion molecular weight of 3,500) and was subjected to a dialysis against distilled water for one week. 150 ml of the resulting polymer solution were treated by freeze-drying in an ordinary manner, whereby 1.60 g of a white solid matter (in the following, denoted occasionally as "acetone-purified product") were obtained (yield 79.2 %).

The results of analyses of this acetone-purified product by ^{1}H-NMR and IR were as given below.

^{1}H-NMR: δ ppm, DMSO-d_6/D_2O = 20/1 (vol.)

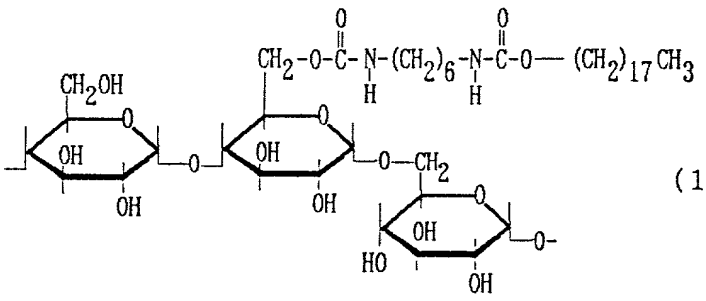
0.86 - 1.70

2.60 - 4.60

4.60 - 5.30

IR (KBr, cm^{-1}): 1680, 1180 - 900

From these data, it was confirmed that the product obtained is a stearyl-pullulan derivative (abbreviated hereinafter sometimes as STP) represented by the following formula (10):



(10)

On the other hand, the above product purified with acetone were analyzed using Preparative TLC in the same manner as in EXAMPLE 1-2, whereby existence of the stearyl dimer represented by the formula (9) was not recognized. Further, the acetone-purified product was analysed by ^{1}H-NMR and the content of the stearyl dimer was calculated from the proton ratio. From the result, existence of the stearyl dimer was not recognized. Therefore the content of the stearyl dimer represented by the formula (9) in the acetone-purified product is 0 % by weight.

From the integration value of the peak area of the ^{1}H-NMR spectrogram, the proportion of introduction of the stearyl groups into pullulan is calculated by the following calculation equation (B):

$$(100 + 2x)/43x = b/a \quad \cdots\cdot (B)$$

in which the symbols denote:

a: the peak area of the stearyl group (δ = 0.86 - 1.70)

b: the peak area of pullulan (δ = 4.60 - 5.30)

x: proportion of substitution with stearyl group per 100 monosaccharide units.

From the calculation, it is found that the proportion of substitution with stearyl group in the stearyl-pullulan derivative is 0.8 group per 100 monosaccharide units.

The acetone-purified product obtained as above was further purified by an ultracentrifugation as in EXAMPLE 1-3 to remove the lower molecular weight pullulan (unsubstituted pullulan). Results are given in Table 6.

Table 6

Content of STP in acetone-purified product (wt. %)	92
Content of STP in ultracentrifug.-purified product (wt. %)	100
Content of unsubstituted pullulan (wt. %)	0
Content of stearyl dimer (wt. %)	0

EXAMPLES 8-1

《 Purification with aprotic polar solvent 》

10 grams of the pullulan-cholesterol derivative obtained in EXAMPLE 1-2 were dissolved in 70 grams of an aprotic polar solvent dimethyl silfoxide (DMSO), whereto were added 1,400 grams of water and the mixture was agitated using a magnetic stirrer for 10 minutes. After the agitation, the mixture was stood still as such at room temperature for one hour. The supernatant was removed by decantation, whereupon the same amount, as extracted, of water was added to the residue and the

resulting mixture was agitated for 10 minutes using a magnetic stirrer, followed by a pause of still stand of one hour. This procedure was repeated twice to effect purification. Then, the lower layer was mixed with 1,000 grams of water, whereupon the mixture was subjected to a freeze-drying over a period of two days. As the result, 7.5 grams of a white solid matter were obtained (yield = 75 %). The results are summarized in Tables 7 and 8.

By adding water to the resulting white solid matter, a 0.2 wt. % aqueous solution was prepared, which was subjected to a ultrasonication using a sonicatore of probe type (TOMY of the firm URP, with a probe outer diameter of 5 mm, at 40 W) for 15 minutes. A sample collected after the ultrasonication was examined by a size exclusion chromatography (SEC) under the conditions given below. The results are shown in Fig. 5. No peak is found in Fig. 5 and, thus, it is seen that a pullulan-cholesterol derivative of high purity in which unsubstituted pullulan had been removed was obtained.

○ Conditions in the SEC analysis:

Apparatus used:		TOSOH HPSEC SYSTEM (trademark, of Tosoh K.K.)
Column	:	TSK-gel G4000SWXL (trademark, of Tosoh K.K.)
Eluent	:	0.02 % NaN_3 in deionized water
Flow rate	:	0.5 ml/min.
Temperature	:	35 °C
Detector	:	RI (differential refractometer)

EXAMPLES 8-2 to 8-6

EXAMPLES 8-2 to 8-6

Purification of each product was conducted using each of the hydrophobic group-containing polysaccharide and the aprotic polar solvent given in Tables 7 and 8 under the purification conditions given in Tables 7 and 6 by the same operation as in EXAMPLE 8-1. In all the purification operations, the procedure of phase separation into two layers was repeated twice. The results are summarized in Tables 7 and 8.

By SEC analysis of each of the hydrophobic group-containing polyasccharides, no peak for the unsubstituted polysaccharide was found in all the EXAMPLES and, thus, it was confirmed that all the products of hydrophobic group-containing polysaccharide had been purified to nearly 100 weight % purity.

Table 7 Results of Acetone-Purified Product

	Example					
	8-1	8-2	8-3	8-4	8-5	8-6
Starting polysaccharide	Pullulan	Pullulan	Pullulan	Pullulan	Mannan	Pullulan
Hydrophobic group	Cholester.	Cholester.	Cholester.	Cholester.	Cholester.	Stearyl
Introduct. proportion [1] of hydrophobic group	1.1	1.3	2.9	1.1	1.9	0.8
Product	CHP	CHP	CHP	CHP	CHM	STP
Content of unreacted polysaccharide (wt. %)	5	10	16	5	20	16
Content of dimer (wt. %)	0	0	0	0	0	0
Purity (wt. %)	95	90	84	95	80	84

Note: 1) Number of groups introduced per 100 monosaccharide units in the product.

Table 8 Purification with Aprotic Polar Solvent and the Results

	Example					
	8-1	8-2	8-3	8-4	8-5	8-6
Amount of acetone-purified product (g)	10	40	5	10	2	3
Aprotic polar solvent						
Kind [1]	DMSO	DMSO	DMSO	DMF	DMSO	DMAc
Amount used (g)	70	320	50	70	12	18
Times weight	7	8	10	7	6	6
Amount of water used (g)	1,400	5,000	800	1,000	150	300
Treating procedures	Water wash Two layer-separation Two steps	Water wash Two layer-separation Two steps	Water wash Two layer-separation Two steps	Water wash Two layer-separation Two steps	Water wash Two layer-separation Two steps	Water wash Two layer-separation Two steps
Treating time (hr)	5	5	4	5	4	4
Yield (wt. %)	75	75	80	72	65	69
Purity (wt. %)	100	100	100	100	100	100
Content of unreacted polysaccharide (wt. %)	0	0	0	0	0	0

Note: 1) DMSO = dimethyl sulfoxide, DMF = N,N-dimethylformamide, DMAc = N,N-dimethylacetamide

In the purification using an aprotic polar solvent, a though-put of purification of 10 grams in about 5 hours was able to be attained and the yield was also superior as it amounted to as high as 65 % by weight or higher. The purification for each product was over within about 2 hours. There is no limitation in principle for the amount to be purified and mass purification can be realized easily. From these points of view, it is seen that the purification with an aprotic polar solvent is very effective for industrial mass production.

INDUSTRIAL APPLICABILITY

The product of polysaccharide containing hydrophobic group obtained by the production process according to the present invention can be utilized as a coating material for coating drug carriers containing encapsulated therein drugs. For example, the product can be used as the coating material for coating drug carriers, such as liposome micrcapsules, microspheres, O/W emulsions and erythrocyte ghost. In this case, the high purity polysaccharide containing hydrophobic group according to the present invention can be used safely as the medical material, since it is present as a high purity product having scarce contents of by-products and of the unsubstituted polysaccharide.